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A STUDY OF MEIOSIS IN THE MICROSPOROCYTES OF INTERSPECIFIC HYBRIDS OF SOLANUM DEMISSUM × SOLANUM TUBEROSUM CARRIED THROUGH FOUR BACKCROSSES[1]

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INTRODUCTION

Many studies on the cytology of *Solanum* hybrids have been undertaken in an attempt to solve the problems of sterility, abnormal segregations, nature of polyploidy, and various other phenomena connected with the breeding of potatoes. Despite the many contributions, little is known of the basic causes of these observed abnormalities.

The present study was undertaken at the suggestion of Dr. Donald Reddick, of Cornell University, who has long been engaged in breeding for blight resistance in the potato by crossing the wild Mexican species *S. demissum* L. (somatic chromosome number=72) with *S. tuberosum* L. (somatic chromosome number=48) in an attempt to retain the commercial characters of *S. tuberosum* and add the blight resistance of *S. demissum* to the stock. This has proved difficult in many ways. With the failure of the expected segregations in the F_2 generation, the backcross method was used, and rigid selection for plants combining the desired characteristics was effected. In general the results were similar to those obtained early in the breeding program by Dr. Reddick and Dr. C. H. Myers, of the Department of Plant Breeding, with species other than *tuberosum*. Their findings hitherto unpublished[3] are as follows:

S. demissum × *S. maglia*
F_1: Plants all immune
F_2: 15 plants, all immune
BC by *maglia*: 19 plants; 10 immune, 9 susceptible

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[2] The writer gratefully acknowledges her indebtedness to Dr. J. R. Livermore, associate professor of plant breeding; Dr. L. W. Sharp, professor of botany; Dr. Donald Reddick, professor of plant pathology, and Dr. L. F. Randolph, professor of botany, Cornell University, for their generous assistance in the course of this work.

[3] Data obtained from Dr. Donald Reddick.

S. demissum × *S. fendleri*
F_1: 8 plants, all immune
F_2: 164 plants, all immune
BC by *demissum*: 37 plants, all immune
BC by *fendleri*: 23 plants, all immune
S. commersonii × *S. demissum*
F_1: 4 plants, all immune
F_2: 32 plants, all immune
BC by *demissum*: 16 plants, all immune

The stage of maturity of the plants did not affect their susceptibility or immunity; all resembled *S. demissum*. More specific data pertinent to the *S. tuberosum* crosses will be presented later.

Plants were available through four backcrosses, and these were used in the present study to see if any clue could be found in the meiotic divisions which would explain the observed breeding behavior. Although several studies have been made on hybrids of *S. tuberosum* and *S. demissum* (*1*, *17*),[4] none known to the writer has included four backcrosses to the *S. tuberosum* parent.

MATERIALS AND METHODS

The species of *Solanum* employed or referred to in this investigation are as follows: *S. tuberosum* Linn.; *S. demissum* Lindl; *S. andigenum* Juz. and Buk.; *S. maglia* Schlecht; *S. commersonii* Dun.; and *S. fendleri* A. Gray. Of these the principal species used were *S. tuberosum* and *S. demissum*.

The somatic chromosome number of *S. tuberosum* as indicated by all of the authors who have determined it, regardless of the variety, is 48, the only exceptions being some South American yellow-fleshed varieties, in which it is 24. Unless otherwise indicated, the varieties used in the backcrosses are all *S. tuberosum* with a chromosome number of 48.

Of this species the following varieties were used: Early Ohio, Jubel, Houma, Katahdin, Ostragis, Russet Rural, Hindenburg, Papa Amarillo ($2n=24$), Arnica, Steinthaler, and Centifolia.

S. demissum is a herbaceous perennial from the mountains of Mexico and is frost-tolerant to 29° F. The somatic chromosome number is 72 (*11*, *18*).

The *S. demissum* originally described by Lindley (*10*) was blight-susceptible, but there are varieties which are blight-resistant, and these were used in the hybrids discussed in this paper. The morphological characters are the same, with the possible exception of the tuber color, which is purple in some varieties.

The *S. demissum* stocks in the plants used in this study are as follows:

Number[5] 886: This stock was obtained from Bukasov.
Number 887: *S. demissum* f. *atrocyaneum* (from Bukasov).
Number 531: Seedling plants only; from El Desierto, Mexico.
Number 533: Same as above; from El Desierto, Mexico.
Number 178: Seed originally from F. W. Keay, Wolverhampton, England.
Number 741: *S. demissum* f. *xitlense* (from Bukasov).
Number 687: *S. demissum* f. *xitlense* from El Desierto, Mexico.

[4] Italic numbers in parentheses refer to Literature Cited, p. 211.
[5] The numbers are those employed by Reddick.

All plants of the parent species and their hybrid progeny from which the collections of buds were made were growing under ideal conditions in the greenhouse during a period when the temperature was seldom above 70° F. They had been planted in February and were illuminated until midnight to induce flowering. Bud collections were made during the months of April and May when the skies at Ithaca were overcast most of the time; this factor is mentioned because sunlight causes greenhouse temperatures to soar, and there has been discussion concerning the influence of temperature on meiotic divisions in *Solanum* (*19*). Seed setting on the greenhouse plants from pollinations made during this period was higher than in former years, which is another indication that conditions were optimum for sexual reproduction in the hybrids. Thus it would seem that the meiotic irregularities observed are not the result of unfavorable environmental conditions, but have their origin in the chromosome complements of the plants.

Buds were collected between 10 and 11 a. m. and fixed for 3 days in Carnoy's fluid (3 parts absolute alcohol:1 part glacial acetic acid). Squash preparations were then made in Belling's acetocarmine without iron. An iron forceps was used to break the anthers, thus supplying the iron. It was found extremely helpful to flame the slides, the chromosomes staining more deeply and the cytoplasm clearing considerably. In many cases, the contents of the sporocytes were emptied into the medium; the relaxation of pressure aided visibility. No disintegration of cytoplasm occurred and the contents of the cell were not disturbed in respect to relative position. A seal of paraffin was applied around the cover slip, and the slides were examined within the next 2 or 3 days. This method proved to be the most efficacious of any attempted, since any procedure used to make permanent mounts seemed to shrink the material and cause clumping, which, with the large number of small chromosomes involved, it was particularly desirable to avoid.[6]

Drawings were made with a Bausch and Lomb binocular microscope having a 10 × ocular and a 98 × fluorite oil-immersion objective, with the aid of a camera lucida. The magnification of the original drawings was approximately 1700 ×.

The drawings are as accurate as it was possible to make them under the difficulties of observation caused by the large numbers of small chromosomes. In some instances it was impossible to decide whether overlapping of chromosomes was being observed or whether actual associations were present. Because of the depth of the nuclei, particularly in the early stages when the chromosomes are scattered, it was not always possible to determine accurately the configurations present, but those included in the plates were unmistakable. The numbers of chromosomes in the polar views varied to such an extent that it was not possible to determine in any way whether an exact count was being obtained; for that reason little stress will be placed upon actual numbers present in the drawings unless certainty was indicated. Root-tip slides were attempted, but because of the large number of chromosomes involved, it was con-

[6] This method was suggested by Dr. L. F. Randolph, following the failure of all others tried by the author.

sidered impracticable to continue the work. In this paper, then, only the behavior of the chromosomes in the meiotic divisions of hybrids through four backcrosses will be discussed.

DESCRIPTION OF RESULTS

The description of results will give mainly the cytological phenomena observed in *S. demissum*, *S. tuberosum*, and their hybrid progeny, together with observations on their characteristic breeding behavior. It will therefore be presented largely as an explanation of the illustrations, more extended comment being reserved for a later section of the paper.

SOLANUM DEMISSUM

Figure 1, *A*, represents an early diakinesis stage in which the nuclear membrane is still present and the chromosomes are not completely contracted. Two chromosomes are in contact with the nucleolus. A few univalents may be distinguished; bivalents and trivalents are in the majority, but quadrivalents and one hexavalent may also be seen. These configurations are characteristic of the chromosomes of *Solanum* at this particular stage of contraction (*2*, *6*, *8*, *11*, *12*). With further contraction and added nucleic acid, the connecting strands which are visible at this stage assume shapes similar to those in figure 5, *F*, which represents a late diakinesis or prometaphase stage.

Figure 1, *B*, shows the alignment of chromosomes on the metaphase plate, with trivalents, bivalents, and an occasional univalent clearly detectable.

Figure 1, *C*, shows an anaphase stage with regularity of chromosome movement indicated.

Figure 1, *D*, represents an interkinesis; the number of chromosomes is unequal in the two nuclei; but, again, bivalents and trivalents comprise the greater number of associations, with one quinquevalent evident. Presumably these associations are not retained in regular divisions. There is some question as to their origin; they may be the result of nonrandom segregation or of a reuniting of ends of chromosomes. Since on the anaphase spindles in *demissum* no evidence of retention of connecting chromatids is indicated, it would seem that a reuniting had occurred. Two nucleoli are visible in the nucleus to the right.

Figure 1, *E*, shows a second anaphase which is proceeding regularly.

Figure 1, *F*, shows the 4 immature spores delimited by walls. No groups of more than 4 spores were found at this stage on any of the slides. Each of the pollen grains would be expected to contain 36 chromosomes if all divisions were regular and segregation of chromosomes were random. The exact number is not represented in the figure shown. In the upper left-hand nucleus, associations of 5 and 7 chromosomes are seen. The remaining 3 nuclei show associations in lesser numbers.

SOLANUM TUBEROSUM

Figure 2, *A–P*, represents three varieties of *S. tuberosum*, one pollen-sterile and two pollen-fertile types.

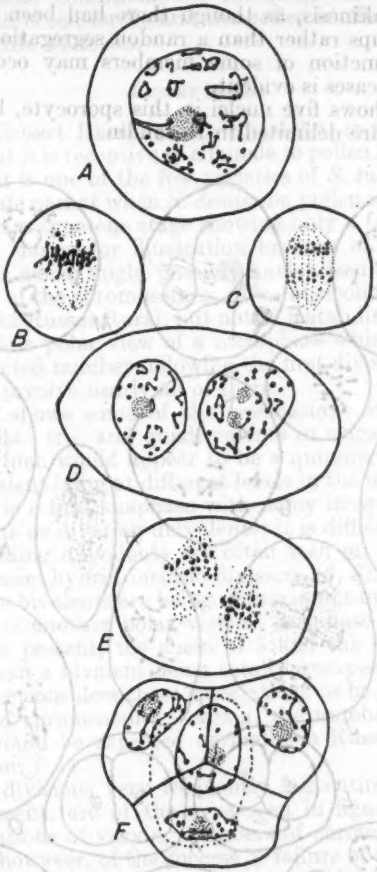

FIGURE 1.—*Solanum demissum: A*, Early diakinesis; *B*, metaphase; *C*, first anaphase; *D*, interkinesis; *E*, second anaphase; and *F*, immature tetrad.

KATAHDIN

Figure 2, *A–D*, inclusive, shows some of the irregularities found in a pollen-fertile variety, the divisions of which for the most part proceed quite regularly with the delimitation of four spores.

Figure 2, *A*, shows an early diakinesis stage with univalents, bivalents, trivalents, and a quadrivalent. Both ring and chain configurations among the tri- and quadrivalent forms are seen.

Figure 2, *B*, is a metaphase in which the chromosomes have for the most part lined up on the plate, but a few univalents and one bivalent have failed to do so.

Figure 2, *C*, is a late anaphase with chromosome bridges in evidence. The chromosomes at either pole have definite connection such as

those seen at diakinesis, as though there had been a segregation of chromosome groups rather than a randon segregation of single chromosomes. Disjunction of some members may occur, but nondisjunction in some cases is evident.

Figure 2, *D*, shows five nuclei in this sporocyte, but for the most part four nuclei are delimited in Katahdin.

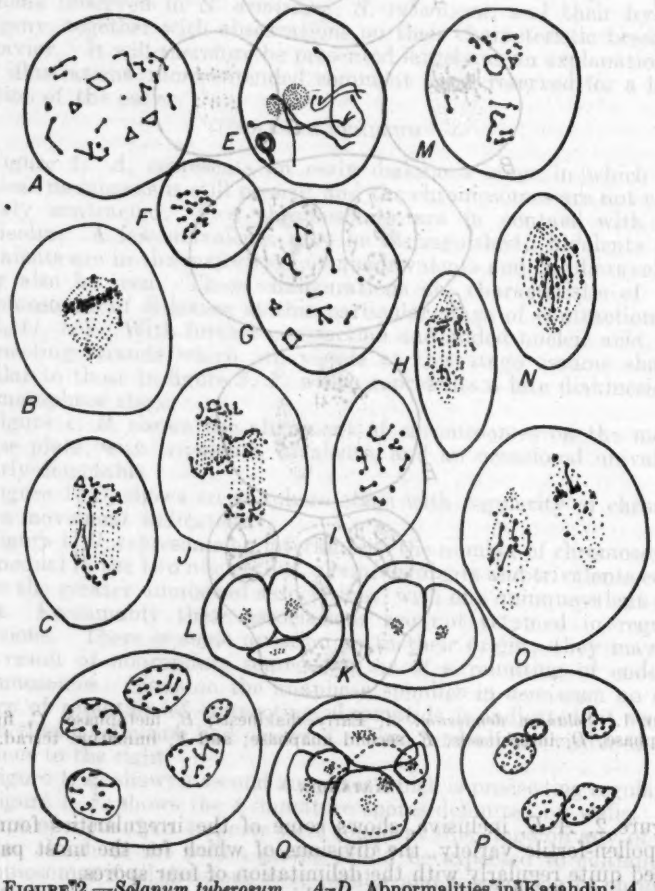

FIGURE 2.—*Solanum tuberosum*. *A–D*, Abnormalities in Katahdin: *A*, Diakinesis; *B*, first metaphase with lagging chromosomes; *C*, late anaphase with bridges and associations; *D*, sporocyte with five nuclei. *E–L*, meiosis in Russet Rural: *E*, Zygotene stage showing two nucleoli and an inversion; *F*, polar view of metaphase; *G*, early diakinesis with associations; *H*, irregular first anaphase; *I*, polar view at anaphase; *J*, irregular second anaphase; *K*, dyad; *L*, triad. *M–Q*, abnormalities in Earlaine: *M*, late first anaphase with associations; *N*, irregular first anaphase; *O*, abnormal second anaphase; *P*, sporocyte with five nuclei; *Q*, six spores including two microspores.

If environmental conditions are favorable for seed setting, the variety Katahdin is self-fertile and the pollen is effective in fertilizing other female-fertile varieties.

Russet Rural

The variety Russet Rural, unlike Katahdin, is almost completely pollen-sterile, but it is receptive as a female to pollen from *S. demissum*. In this respect it is one of the few varieties of *S. tuberosum* favorable for use as a female parent when *S. demissum* pollen is used.

Figure 2, *E*, is a zygotene stage showing only a few of the chromosomes. It was chosen for illustration because of the configuration at the lower left, which might possibly have arisen as the result of an inversion in one of the chromosomes. Two nucleoli have been formed; this is frequent in Russet Rural, but not in Katahdin and Earlaine.

Figure 2, *F*, is a polar view of a metaphase which has 24 chromosomes, the expected number following the first division. Associations may be seen to involve nearly all of them.

Figure 2, *G*, shows some of the associations of early diakinesis, including uni-, bi,- tri-, and quadrivalents in rings and chains. The configuration which would appear to be a quinquevalent is in reality a bi- and a trivalent lying at different levels in the nucleus.

Figure 2, *H*, is a first anaphase with many irregularities, including lagging bivalents or dividing univalents; it is difficult to say which is the case. Dividing univalents are often seen on the first anaphase spindle in *Solanum* hybrid meiotic divisions (*6*, *8*). It would appear that some of the bivalents are being drawn intact to either pole.

Figure 2, *I*, is another polar view at anaphase chosen because of the associations present, the chain of 5 and the evident association of a trivalent and a bivalent being totally unexpected in a tetraploid individual if one considers these associations to be secondary and due to homology of chromosomes. Again, the number of chromosomes is 24, which would be expected normally in Russet Rural following the first division.

The second divisions very frequently fail entirely in this variety, and, when present, are of the type seen in figure 2, *J*, with very evident associations of varying numbers of chromosomes.

Regardless, however, of the success or failure of the second division, the pollen that is formed appears to be entirely devoid of functional cytoplasm, although shrunken protoplasm can usually be distinguished in some portion of the pollen grain. Dyads, triads, and higher, groupings are present, but all are functionless. Figure 2, *K* and *L*, shows a dyad and a triad, respectively.

Earlaine

Figure 2, *M*, *Q*, inclusive, are from the variety Earlaine.

Figure 2, *M*, is a late first anaphase approaching interkinesis in which a ring of seven chromosomes is visible. The bivalent to the left will probably be excluded from the nuclei.

Figure 2, *N*, is an early anaphase with evidence of difficulty in breakage of the connecting chromatids; one member of the group remained at the pole or was precocious in movement.

Figure 2, *O*, is a second anaphase showing about the same type of abnormality. These chromosomes were on the upper side of the spindle, but, presumably, were the other side in evidence, the same types of irregularity would be observed.

Figure 2, *P* and *Q*, are of nucleus formation and pollen groups, respectively. One micropollen grain is present in *P* and two micrograins in *Q*; yet, for the most part, Earlaine has good functional pollen.

FIRST GENERATION HYBRIDS

Breeding Behavior

Speaking of the first-generation hybrids, Reddick (*15, p. 556*) states:

> At Ithaca, thus far, *S. demissum* as pollen parent has given a very scant set of seeds only 5 times and each time on variety Rural or on Rural hybrids. The first-generation hybrids are intermediates in many characters, are all blight-immune, and are practically male-sterile. Less than 100 individuals of the second generation have been produced. A segregation occurs and blight immunity is found to be heritable, although it is not possible to determine the probable mode from such meager material. Also, some of the progeny have the general appearance of the Rural parent with fairly short stolons and tuber production before autumnal equinox.
>
> When *Solanum demissum* is the female parent and fertile pollen from cultivated varieties is employed, the first-generation hybrids usually resemble the wild parent rather closely, although some intermediate characters may be observed. Practically all of the plants of this generation are immune from blight (90 per cent at present, but some of the *demissum*-like parents are of questionable genetic purity), they develop long to very long stolons, and a good many of them persist in the habit of producing tubers only with short day. Such hybrids are mostly fertile. In the second and succeeding generations (at least as far as the fourth) all plants tested have reverted to the wild type; all are immune from blight and continue so in succeeding generations.

The following excerpt is taken from a later paper of Reddick (*16, pp. 121–122*):

> After a first cross has been effected there comes the problem of selfing some of the first generation hybrids. This is often very difficult or quite impossible to accomplish. Many such plants are self sterile. The easiest method is to plant a considerable number of seeds and search for plants which produce seeds in the field. Such seeds may be used safely since they are almost certainly self-fertilized; and in any event the result is almost always disappointing. The recombination and segregation of characters which ordinarily are expected rarely occurs. Instead, the second generation usually has the appearance and characters of *S. demissum*, the female parent of the first cross.

Because of the desirability of having some knowledge of the performance of the plants included in this study, the following notes from the field and greenhouse as taken by Reddick and Peterson are included:

FFA–1: [*S. demissum* (886)] × [860 (Early Ohio)]

Thirty-five immune plants were obtained, all of which were large and late except 1 or 2, which died early. The plants are dark green, drought-tolerant, and also frost-tolerant to 20° F. They bear large white tubers. In a breeding test, 2 pollinations set seed and 2 failed.

FEJ–1: [*S. demissum* (531)] × [245/25 (U. S. 444/12 × Jubel)]

Fifteen immune plants were secured from this cross, all of which were drought-tolerant. Some were frost-tolerant, but most of them frosted. None of the tubers were purple early, but by December all but 2 were purple. In 1944 in the greenhouse 2 pollinations set seed and 6 failed. In 1946, 1 pollination was successful.

FEK–1: [*S. demissum* (531)] × [245/86 (U. S. 444/12 × Jubel)]

This plant set seeds naturally in the field, but was unsuccessful as a male in crosses. It failed to set seed in 1944. In 1946, there were one set and six failures. The plants are drought-tolerant and excellent in appearance.

FEL–1: [*S. demissum* (531)] × [627/126 (Houma×Katahdin)]

Seventeen immune plants were obtained from this cross; 12 tubers produced large plants with a big crop of tubers ranging in size from eggs to marbles. The plants are drought-tolerant and late. In 1944 they failed 6 pollinations and set 1. In 1946 they failed 7 pollinations and set 5.

FEM–1: [*S. demissum* (531)] × [902/4 (*S. andigenum*)]

S. andigenum is a 48-chromosome South American species. The F_1 set seeds in the field. In 1944 3 pollinations failed. In 1946 there were 2 sets and 7 failures. The plants are pale green. Some tubers are tinged with purple. No scab was present. All plants were frost- and drought-tolerant.

FEQ–1: [*S. demissum* (531)] × [CDU/4 (Houma × Ostragis)]

Twelve immune plants were secured. In 1944 this plant set 1, failed 2 pollinations; in 1946 it set 1 and failed 4. Most of the plants are frost-tolerant, but there is variability. Most of the tubers have a purple tinge, but only white tubers were saved.

FEY–1: [*S. demissum* (886) × [245/25 (U. S. 444/12 × Jubel)]

Twenty-five immune plants were obtained. FEY–1 sets seeds naturally in the field. In 1944 it set 2 and failed 3 pollinations; in 1946 it set 5 and failed 5. The pollen from this plant will set on Rural. It is drought- and frost-tolerant with very long stolons and large tubers.

FFC–1: [*S. demissum* (886)] × [CDU/4 (Houma × Ostragis)]

Twelve immune plants were obtained which all looked alike in the field. All had white tubers and long stolons. The plants were large and dark green and drought- and frost-tolerant. In 1944 pollination with 5 types of pollen resulted in failure and 2 were successful. In 1946 the 1 pollination performed was a failure.

FFD–1: [*S. demissum* (886)] × [Jubel]

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Five plants were obtained, four of which were immune and one susceptible. Three plants were large and green with white tubers and were frost- and drought-tolerant. They were barren in the greenhouse. In 1946, pollination with four types of pollen was effective and five were not.

Cytology

Figure 3, *A* (FEM–1), *B* (FEK–1), *C* (FEY–1), and J (FFA–1) are prophase stages. In *B* and *C* there are 2 nucleoli, a condition which is rather unusual in these hybrids. In *C* the mode of pairing on the right may indicate a translocation in 1 of the chromosomes. Figure 3, *B*, is a diakinesis stage, and *A* and *J* are an early diakinesis stage in which there is considerable variation in pairing. Figure 3, *J*, has many bivalents, whereas *A* has mostly higher degrees of valency. The plant from which *A* was derived is pollen-fertile, but the plant forming mostly bivalents is pollen-sterile. In no case have 12 univalents and 24 bivalents been seen, as was reported by Salaman (*17*) for his material. This would lead one to believe that pairing may take place not only between the *tuberosum* sets and the *demissum* sets, but also between chromosomes from the same parent, presumably the one which contributes the larger number of homologues. Cytological chromosome markers would be needed to decide this definitely.

Figure 3, *D* (FFA–1), *E* (FEJ–1), *G* (FFD–1), and *F* (FEJ–1) show successive stages in metaphase I and anaphase I. A polar view with 27 chromosomes is seen in figure *F*. If the 60 chromosomes present in the hybrid had been equally divided, 30 chromosomes would be expected in this polar view. Figure 3, *D*, shows several chromosomes out of the metaphase plate. The size of the chromosomes is interesting when compared with that in figure 3, *E*, for instance. The magnification is the same, yet there is an appreciable difference in size. Fewer associations are present in *E* than in *D*. Ellison believed that the diameter or size of the bivalents may be altered by the general genetical composition of the plant, or as a result of the effect of the environment on the plant. He states (*2, pp. 248–249*):

> The fact that the bivalents in the fertile varieties are of less diameter than in the self sterile ones is difficult to explain. . . . Moreover, since the self sterile varieties are so, either because of their genetical composition or the effect of the environment or both, it is equally feasible that the diameter or size of the bivalents may be altered by the general genetical composition of the plant, or as a result of the effect of environment upon the plant. In either case the effect is the same in that of the cases examined self sterility was associated with an increase in the diameter of the bivalent.

It is quite evident that there is a difference in size between the chromosomes of the F_1 plants, which are mostly pollen-sterile, and the third backcross plants which are mostly pollen-fertile except the chromosomes in figure 3, *H*, which is an unreduced cell.

Figure 3, *G*, has a ring configuration which is seldom seen in this material. The chromosomes are scattered over the spindle, and the prophasic associations appear to have been retained; that is, disjunction has been incomplete. The same is true of figure 3, *N*. What appears to be a quinquevalent is a bi- and a trivalent lying in close proximity. There is a striking contrast in size between the inter-

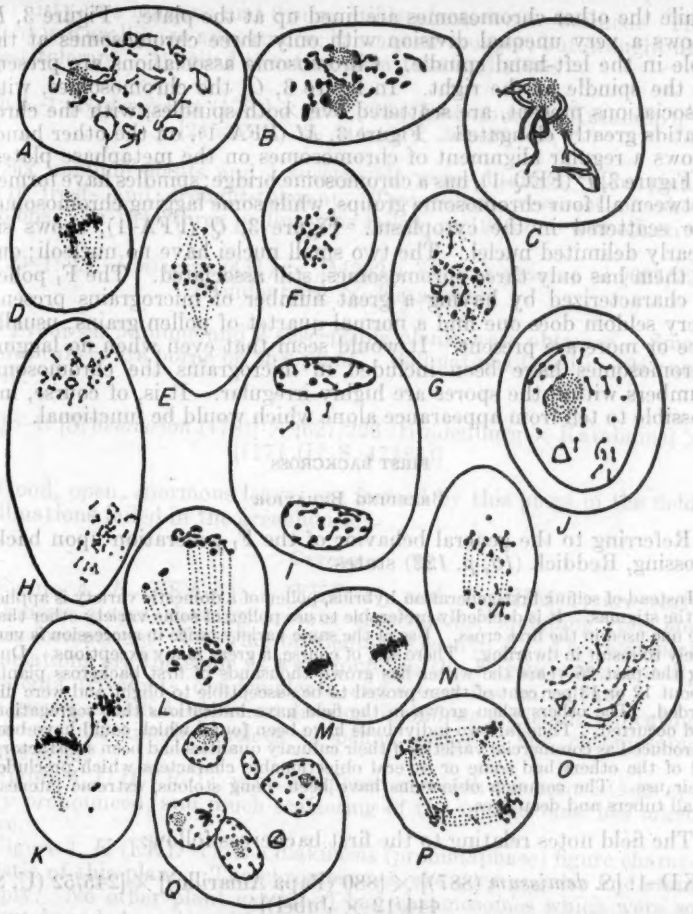

FIGURE 3.—First-generation hybirds: *A*, FEM–1, Early diakinesis; *B*, FEK–1, early diakinesis with two nucleoli; *C*, FEY–1, zygotene with two nucleoli; *D*, FFA–1, irregular first metaphase; *E*, FEJ–1, first metaphase with few associations; *F*, FEJ–1, polar view of metaphase; *G*, FFD–1 first metaphase; *H*, FEL–1, unreduced chromosome groups; *I*, FEQ–1, interkinesis; *J*, FEL–1, unreduced cell; *K*, FEJ–1, irregular second anaphase; *L*, FFC–1, abnormal second anaphase; *M*, FFA–1, regular metaphase; *N*, FEJ–1, irregular first anaphase; *O*, FEL–1, irregular second anaphase; *P*, FEQ–1, chromosome bridges and associations at late anaphase; *Q*, FFA–1 sporocyte with six nuclei.

kinesis figure of *I* and the unreduced chromosome groups in *H* (FEL–1). The former cell will form a dyad. In *T* (FEQ–1) three chromosomes have been excluded from the nuclei.

Figure 3, *K* (FEJ–1), *L* (FFC–1), and *O* (FEL–1) are different stages in second anaphase showing various abnormalities. Figure 3, *K* has a trivalent stretched between the two spindles and three univalents in the cytoplasm. One univalent is at the pole in the spindle to the left,

while the other chromosomes are lined up at the plate. Figure 3, *L*, shows a very unequal division with only three chromosomes at the pole in the left-hand spindle. Chromosome associations are present in the spindle at the right. In figure 3, *O*, the chromosomes, with associations present, are scattered over both spindles, with the chromatids greatly elongated. Figure 3, *M* (FFA–1), on the other hand, shows a regular alignment of chromosomes on the metaphase plates.

Figure 3, *P* (FEQ–1), has a chromosome bridge; spindles have formed between all four chromosome groups, while some lagging chromosomes are scattered in the cytoplasm. Figure 3, *Q* (FFA–1), shows six clearly delimited nuclei. The two small nuclei have no nucleoli; one of them has only three chromosomes, still associated. The F_1 pollen is characterized by having a great number of micrograins present. Very seldom does one find a normal quartet of pollen grains; usually five or more are present. It would seem that even when no lagging chromosomes have been included in micrograins the chromosome numbers within the spores are highly irregular. It is, of course, impossible to tell from appearance alone which would be functional.

FIRST BACKCROSS

Breeding Behavior

Referring to the general behavior of the F_1 generation upon backcrossing, Reddick (*16, p. 122*) states:

> Instead of selfing first-generation hybrids, pollen of a domestic variety is applied to the stigmas. It is decidedly preferable to use pollen of some variety other than the one used in the first cross. Use of the same variety twice in succession is very likely to result in dwarfing. There are, of course, a great many exceptions. During the past 25 years the writer has grown thousands of first backcross plants. About 12 or 13 per cent of them proved to be susceptible to blight and were discarded. The others when grown in the field gave indications that segregations had occurred. Thus far two individuals have been found which could have been introduced as commercial varieties if their culinary qualities had been satisfactory. All of the others had some or several objectionable characters which precluded their use. The common objections have been—long stolons, extreme lateness, small tubers and deep eyes.

The field notes relating to the first backcross follow:

EKD–1: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [245/52 (U. S. 444/12 × Jubel)]

"Papa Amarillo" is probably a diploid South American yellow-fleshed variety of *S. tuberosum*. The plant failed to set seed in 1944 and 1946.

EVR–1: [*S. demissum* (178)] × [Ostragis] × [245/52 (U. S. 444/12 × Jubel)]

Fifty-seven immune and 11 susceptible plants were obtained. The plants had a fairly good appearance in the field. They bore small tubers on long stolons. No pollen was effective in seed production in 1946.

EVT–2: [*S. demissum* (178)] × [Ostragis] × [627/226 (Hindenburg × Katahdin)]

Thirty-two immune and 8 susceptible plants were obtained. This plant is Rurallike in appearance; it is a poor cropper, but drought-and frost-tolerant. It failed to set seeds in 1944 and 1946.

FKA–1: [*S. demissum* (178)] × [627/226 (Hindenburg × Katahdin)] × [245/25 (U. S. 444/12 × Jubel)]

Six immune plants and 1 susceptible were obtained. The plants were frost- and drought-tolerant. Two tubers were set on short stolons. In 1944 three pollinations failed; in 1946 one was effective.

FLD–1: [*S. demissum* (431) × [M262/1 (*S. andigenum*) × [627/226 (Hindenburg × Katahdin)]

Seventeen immune and 2 susceptible plants were obtained. Small white tubers are borne on this plant. Pollinations failed in 1945 and 1946.

FQS–1: [*S. demissum* (178)] × [627/226 (Hindenburg × Katahdin)] × [1171 (U. S. 47285)]

Good, open, enormous leaves are formed by this plant in the field. Pollinations failed in the greenhouse.

Cytology

Figure 4, *B* (FKA–1), *I* (FKD–1), and *J* (EHM–1) are early diakinesis stages showing various associations, including ring and chain formations. Two ring bivalents are seen in *B*. The remaining associations are the same as were found in the F_1 generation; mostly bivalents, trivalents, quadrivalents, and a very few univalents.

Figure 4, *A* (EKA–1), a first division, includes a nucleolus, indicating that an interkinetic nucleus is being formed. The chromosome count is 33.

Figure 4, *C* (EVR–1), is an anaphase in which the associations are very pronounced, and much scattering of the smaller ones has taken place.

Figure 4, *K* (EKD–1), is a diakinesis (prometaphase) figure characteristic of this plant. The chromosomes are very angular and stain deeply. No other plant examined had chromosomes which were so definitely rod-shaped at this stage.

Figure 4, *L* (FLD–1), is an anaphase with a ring configuration as well as a T chromosome in the center right of the figure. T chromosomes are characterized by having a satellite end (*13*). Lamm (*8*) reports their presence in the species of potatoes which he examined. They will be treated further in the discussion. Few were found in this material.

Figure 4, *N* (EHM–1), is a second anaphase which is proceeding irregularly with groups of chromosomes segregating and retaining their connections. Some connections in the spindle to the right have been broken and the dangling ends can be seen.

Figure 4, *O* (EHM–1), is a polar view of a second division. One group has practically all the chromosomes associated, whereas the other group has relatively few.

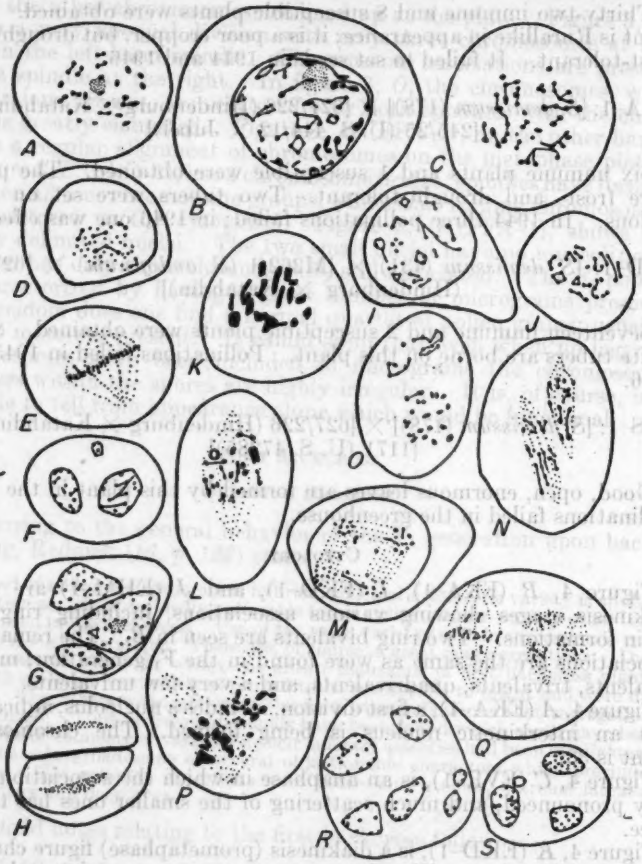

FIGURE 4.—First backcross: *A*, EKA–1, polar view, interkinetic nucleus forming; *B*, FKA–1, early diakinesis; *C*, EVR–1, anaphase with pronounced associations; *D*, CTT–1, polar view of late anaphase (ameiotic division); *E*, CTT–1, metaphase plate (ameiotic division); *F*, CTT–1, interkinetic stage with a micronucleus; *G*, CTT–1, sporocyte with three nuclei; *H*, CTT–1, dyad; *I*, FKD–1, early diakinesis; *J*, EHM–1, early diakinesis; *K*, EKD–1, prometaphase with angular chromosomes; *L*, FLD–1, first anaphase with ring configuration; *M*, EKD–1, late second anaphase with bridges; *N*, EHM–1, irregular second anaphase; *O*, EHM–1, polar view of a second division; *P*, EKD–1, fused second anaphase spindles; *Q*, EIU–2, second anaphase with four spindles; *R*, EIU–2 sporocyte with six nuclei; *S*, FQS–1, sporocyte with three regular nuclei and one micronucleus.

Figure 4, *M* (EKD–1), is a late second anaphase with a bivalent stretched between the lower two nuclei to form a chromosome bridge which has not broken. Association of chromosomes in bi-, tri-, and quadri-valents is seen.

Figure 4, *P* (EKD–1), shows fused spindles with the chromosomes appearing almost as they did at diakinesis.

Figure 4, *Q* (EIU–2), is a second division with three spindles having formed where one would be expected in the right-hand portion of the cell. In the spindle to the left all but four bivalents have lined up on the metaphase plate.

Figure 4, *R* (EIU–2), and *S* (FQS–1) show how irregular the formation of pollen grain nuclei can be. The micronucleus in *S* contains only two chromosomes.

Figure 4, *D-H* are from CTT–1, which had Rural as the female parent. Figure 4, *D* and *E* are stages in ameiotic divisions which will lead to dyad formation. About one-half of the pollen formed is the result of divisions such as these.

Figure 4, *F*, is an interkinesis figure with a micronucleus containing five chromosomes.

Figure 4, *G*, shows three nuclei. Many triads as well as higher grouping of pollen are formed in CTT–1. None of its pollen is functional; in this respect it is like Rural, the female parent. It also resembles Rural in being a functional female. This would seem to indicate that a maternal influence is exerted on the progeny.

SECOND BACKCROSS

Breeding Behavior

Regarding the behavior of the second back-cross plants, Reddick (*16, p. 122*) remarks:

> If one selects some of the more satisfactory backcrosses and backcrosses again to a domestic variety, a progeny is obtained, with more failures than successes, of which about one-third are susceptible. The plants which do not blight, when grown in the field, have the gross appearance of a field of commercial potatoes. Plants can be found which are mature before the autumnal equinox, and which bear 2, 3, or even 4 pounds of tubers of commercial size. Several thousand selections of plants in this stage have been made and tested. At the present time not one of them has been retained. They have been rejected for various reasons but perhaps the commonest cause of rejection has been deep eyes or irregular contour.

Field notes on the individual plants selected in the present study follow:

EML–1: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [860 (Early Ohio)] × [245/25 (U. S. 444/12 × Jubel)]

The tubers have a pink tinge but are smooth and fleet-eyed. Very few are of commercial size, and the stolons are quite long. Reddick comments that this plant is "still too wild." It failed to set seed in 1944 and 1946.

EWM–3: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [1144 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)]

Thirty-three susceptible and 22 immune plants were obtained from this cross. The immune plants were late and remained green until frost. The tubers were white and 5 of them were of commercial size. Seeds were set in the field and in the greenhouse in 1944, but in 1946

the plant bloomed too late to escape the heat in the greenhouse; the one pollination attempted resulted in failure.

EXW–2: [*S. demissum* (533)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [627/226 (Hindenburg × Katahdin)]

Forty-six susceptible and 35 immune plants resulted from this cross. They bore white tubers of noncommercial size and died early. The plant is drought-tolerant. Greenhouse pollinations failed.

EYA–1: [*S. demissum* (533)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [627/226 (Hindenburg × Katahdin)]

The plants were late and bore six to eight tubers of commercial size. They set seed very infrequently after pollination.

FSK–1: [*S. demissum* (531)] × [883 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)] × [1065 (Arnica)]

This plant is scab-resistant. In 1946 it set no seed because it was very late in blooming.

FSU–1: [*S. demissum* (741)] × [883 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)] × [DLD–7 (Jubel × F_2 of 883)]

This was one of seven transplants. It bore white, oval, smooth, fleet-eyed tubers of commercial size. Three pollinations produced seed and three failed to do so in 1946.

ESO–1: [Rural] × [*S. demissum* (687)] × [1063 (Ostragis)] × [245/52 (U. S. 444/12 × Jubel)]

Thirty-six immune and 36 susceptible plants were obtained from this cross. The plant is Rurallike and early; it is not frost-tolerant. The tubers have yellow skin and are smooth and double-fleet with a pink dotted eye probably of Ostragis origin.

Cytology

Figure 5, *A* (EML–1), shows one chiasma at top and perhaps others. It is possible that a piece had been translocated between the chromomeres in the large synaptic configuration in the right of the figure, and that this led to an interrupted pairing. It is difficult to tell whether the point of crossing in the lower configuration is in reality a chiasma or whether the position of the chromosomes is such that it only appears to be, particularly since the chromosomes are definitely unlike in morphology.

Figure 5, *B* (EML–1), is a later stage. Chain associations are prevalent in this nucleus.

Figure 5, *C* (ESO–3), has a predominance of bivalents.

Figure 5, *F*, is a true diakinesis of EML–1. The chromosomes are very large in comparison with those of earlier stages. Few slides with cells in this particular stage were seen. There appear to be only two univalents. The number of chromosomes in the central configuration is impossible to determine.

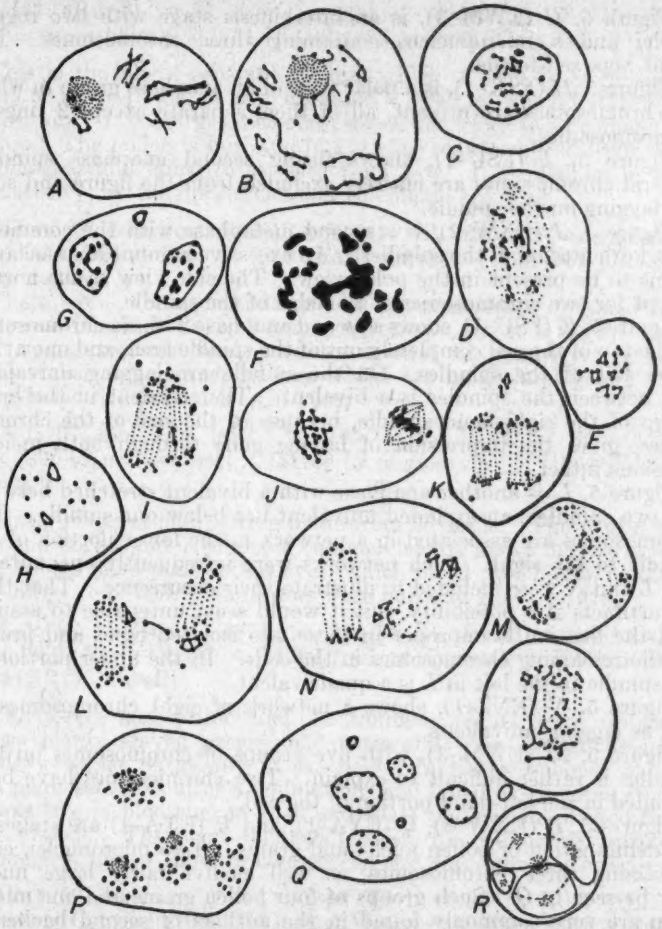

FIGURE 5.—Second backcross: *A*, EML–1, Prophase showing chiasma; *B*, EML–1, early diakinesis; *C*, ESO–3, early diakinesis; *D*, EYA–1, irregular first anaphase; *E*, FSK–1, polar view of first anaphase; *F*, EML–1, true diakinesis; *G*, EWM–3, interkinesis with micronucleus; *H*, FSK–1, polar view of anaphase; *I*, FSU–1, fused second anaphase spindles; *J*, EXW–2, second metaphase; *K*, FSU–1, irregular second anaphase; *L*, ENZ–1, irregular second anaphase; *M*, EWM–3, second anaphase with five groups of chromosomes; *N*, ENZ–1, second anaphase with associated chromosomes; *O*, EWM–3, first anaphase with bridge; *P*, EXW–3, regular nucleus formation; *Q*, EYA–1, irregular nucleus formation; *R*, EYA–1, five spores including a microspore.

Figure 5, *D* (EYA–1), is a first anaphase in which most of the chromosomes are either passing without disjunction to the poles or have never been regularly oriented at the equator.

Figure 5, *E* (FSK–1), is a polar view of an anaphase with bi-, tri-, and quadrivalents present; 24 chromosomes are visible.

Figure 5, *G* (EWM–3), is an interkinesis stage with two regular nuclei and a micronucleus containing three chromosomes. This plant was self-fertile.

Figure 5, *H* (FSK–1), is a polar view of an anaphase group in which 22 chromosomes are present, all of them separate except 2 rings of 3 chromosomes.

Figure 5, *I* (FSU–1), shows fusing second anaphase spindles. Several chromosomes are entirely excluded from the figure and some are lagging on the spindle.

Figure 5, *J* (EXW–2), is a second metaphase with the commonly found orientation of the spindles. An excessive amount of association seems to be present in the polar view. The side view seems normal except for two chromosomes at the edge of the spindle.

Figure 5, *K* (FSU–1), shows a second anaphase with six chromosomes at the top of the cell completely out of the spindle area, and one at the lower left of the spindles. On the spindle are lagging univalents and between the spindles is a bivalent. The trivalent in the lower group of the right-hand spindle, because of the size of the chromosomes, gives the impression of having gone through both meiotic divisions intact.

Figure 5, *L*, is another anaphase with a bivalent stretched between the two spindles; an excluded univalent lies below one spindle. The chromosomes are associated in a network in the lower portion of the spindle to the right. Such networks were infrequently encountered and *L* and *N* were included to illustrate their occurrence. That they are artifacts is a possibility, but it would seem untenable to assume that the fixation is improper in these two isolated cases and proper for the remaining chromosomes in the cells. In the upper portion of the spindle to the left in *L* is a quadrivalent.

Figure 5, *N* (ENZ–1), shows a network of eight chromosomes as well as lagging univalents.

Figure 5, *M* (EWM–3), with five groups of chromosomes on two spindles is rather difficult to explain. Two chromosomes have been excluded in the left-hand portion of the cell.

Figures 5, *P* (EXW–3), *Q* (EYA–1), and *R* (EYA–1) are stages in the delimination of pollen nuclei and grains. Two micronuclei, each containing three chromosomes, as well as five fairly large nuclei may be seen in *Q*. Such groups of four pollen grains and one micrograin are very commonly found in the anthers of second backcross individuals.

THIRD BACKCROSS

Breeding Behavior

In regard to the third backcross Reddick (*16, p. 122*) says:

> Since a high proportion of the plants in the second backcross still carry the gene for immunity, a third backcrossing has been tried. About one-half of the progeny continues to give immune reaction by inoculation and in this generation many more desirable types can be found and tests of these, which are now in progress, indicate that suitable types have been isolated which have enough of the desirable horticultural characteristics to make them satisfactory commercial varieties.

Field notes on the plants examined in the present study follow:

CRH–7: [Rural] × [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)]

This is the blight-resistant Empire which is soon to be released. From 50 seeds 16 plants were obtained in 1940, of which CRH–7 was a selection. It is a large green plant which bears four to six commercial tubers. The pollen is fair, and in 1946 the plant set seed from three pollinations and failed to set seed from two. It is medium in time of maturity; by the first of October the plants are dead.

DGH–7: [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)] × [528/274 (Jubel × U. S. 44537)]

There were 178 susceptible and 90 immune plants obtained from this cross of which DGH–7 was a selection. It has a rugose leaflet and is an excellent plant, medium in time of maturity. It bears many tubers of commercial size which are mealy and good. This plant failed five pollinations in 1946.

EOJ–1: [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [890 (*S. andigenum*)] × [M166 (*S. andigenum*)] × [1064 (Hindenburg)]

Thirty-six transplants were made from 50 seeds. The plants were late and bore about 7 tubers of commercial size and 8 the size of eggs. The tubers have pink-dotted eyes, which in all probability is due to the influence of 890. No flowers were borne in 1944 or 1946.

FIX–1: [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [245/25 (U. S. 444/12 × Jubel)]

Thirty-one susceptible and 11 immune plants were obtained. These plants stayed green until frost and were drought-tolerant. Seeds were set natuarally in the field. In the greenhouse in 1945 this plant set seeds after 3 pollinations and failed to set seeds after 3. It was late in blooming in 1946 and the 1 pollination effected failed to set seeds. The plant is much too late for commercial purposes, although it bore 1 tuber of commercial size and 5 the size of eggs.

FMP–1: [*S. demissum* (533)] × [860 (Early Ohio)] × [1067 (Hindenburg × Centifolia)] × [OE/1 (F_2 of Metzger's seedling 883)] × [245/25 (U. S. 444/12 × Jubel)]

Fourteen susceptible and 25 immune plants were obtained. FMP–1 set seeds in 1944 in the field. It is drought-tolerant and scab-resistant. Seven pollinations failed and one succeeded in 1946. The tubers are largely noncommercial.

Cytology

Figure 6, *A* (CRH–7), is an early diakinesis stage with uni-, bi-, tri-, and quadrivalents.

Figure 6, *B* (DGH–7), is a polar view of a first metaphase in which 24 chromosomes can be distinguished. One association of 5 and 6 associations of 2 chromosomes are seen.

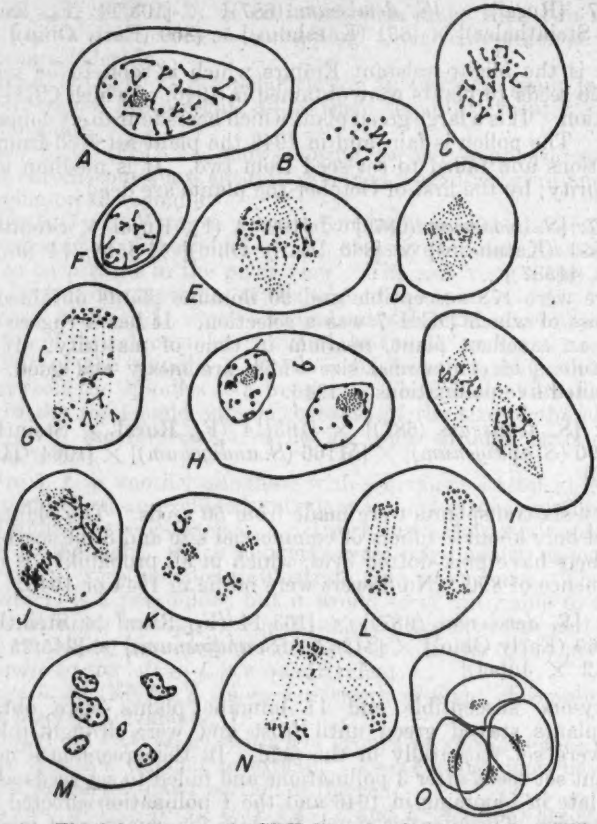

FIGURE 6.—Third backcross: *A*, CRH–7, early diakinesis; *B*, DGH–7, polar view of first metaphase; *C*, CRH–7, polar view of ameiotic metaphase; *D*, DGH–7, first metaphase; *E*, CRH–7, early anaphase; *F*, FIX–1, early interkinesis (one nucleus); *G*, FIX–1, irregular late anaphase; *H*, EOJ–1, interkinesis figure with micronucleus; *I*, CRH–7, second anaphase showing association of chromosomes; *J*, EOJ–1, second anaphase with associated chromosomes; *K*, FMP–1, five groups of chromosomes; *L*, DGH–7, second anaphase; *M*, FIX–1, six nuclei in sporocyte; *N*, CRH–7, three groups of chromosomes in sporocyte; *O*, DGH–7, normal quartet of spores.

Figure 6, *C* (CRH–7), is a polar view of an ameiotic metaphase division which will give rise to a dyad. Fifty-seven chromosomes are countable and associations of 2, 3, 4, 5, and 7 are present. This affinity is definitely not due to primary association, because pairing evidently did not take place. CRH–7 had Rural as the female plant, and the dyad formation characteristic of Rural is evident.

Figure 6, *D* (DGH–7), is a metaphase which is remarkable because of the small size of the chromosomes. The number would lead one to believe that they are typical bivalents, but the size would indicate that no pairing had taken place. Ellison (*2*) believes that the size

difference between the chromosomes of fertile and sterile individuals is significant.

Figure 6, *E* (CRH–7), is an early anaphase in which a great difference in size of chromosomes is apparent. Some of those present are evidently true bivalents; others have not paired.

Figure 6, *F* (FIX–1), is an early interkinesis with 26 chromosomes associated in bi- and trivalents.

Figure 6, *G* (FIX–1), is a late anaphase with many irregularities, including an excluded bivalent, an excluded trivalent, lagging univalents, and one quadrivalent which possibly would be included in the lower nucleus.

Figure 6, *H* (EOJ–1), is an interkinesis figure with a quadrivalent and two univalents, one divided, free in the cytoplasm. In the nucleus to the right, a T chromosome is visible just below the nucleolus.

Figure 6, *I* (CRH–7) and *J* (EOJ–1) are second anaphases with a great deal of association and segregation of groups of chromosomes taking place.

Figure 6, *L* (DGH–7), is a regular anaphase except for one lagging chromosome.

In figure 6, *N* (CRH–7), three groups of chromosomes have been formed, with one chromosome completely out of the spindles.

Figure 6, *K* (FMP–1), shows five groups of chromosomes with an association of six in the form of a chain in the upper left-hand group. One chromosome is free in the cytoplasm and will evidently not be included in a nucleus.

Figure 6, *M* (FIX–1), demonstrates the delimitation of six nuclei, one of which has only four chromosomes.

Figure 6, *O* (DGH–7), shows a normal quartet of spores. DGH–7, CRH–7, FIX–1, and FMP–1 are all self-fertile plants with pollen that is sufficiently good to effect pollination in functional females of other varieties.

FOURTH BACKCROSS

Breeding Behavior

No published account of the general behavior of the fourth backcross is available, but the following field notes indicate the characteristics of the group:

FAU–1: [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [851 (Katahdin)] × [OU/3 (F_3, Rural × Steinthaler)] × [528/224 (Jubel × U. S. 44537)] × [627/226 (Hindenburg × Katahdin)]

From this cross 33 susceptible and 163 immune plants were derived. Jubel and Hindenburg both have a certain amount of immunity. The plants are excellent and erect under field conditions. The tubers are white, oval, smooth, and fleet; 3 large ones were borne by this plant in 1944. No seeds were set in 1946.

FNH–1: [M470 (*S. andigenum*)] × [687 (*S. demissum*)] × [821 (2bc, *S. demissum* × *S. maglia* × Silhonetto × 349)] × [860 (Early Ohio)] × [1063 (Ostragis)] × [245/25 (U. S. 444/12 × Jubel)]

This is a selection from a cross of a fertile immune on *S. andigenum*. The plant sets seeds naturally in the field. In 1944 it produced

two tubers of commercial size and three the size of walnuts. No seeds were set in the greenhouse in 1946.

FQE–2: [*S. demissum* (687)] × [103/14 (F_2, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)] × [528/118 (Jubel × U. S. 44537)] × [1067 (Hindenburg × Centrifolia)].

This plant was one of 21 transplanted. It is erect and of excellent size in the field. The tubers are white, oval, smooth, and fleet-eyed; 1 the size of an egg and 6 the size of walnuts were obtained in 1945. No seeds were set in the greenhouse in 1946.

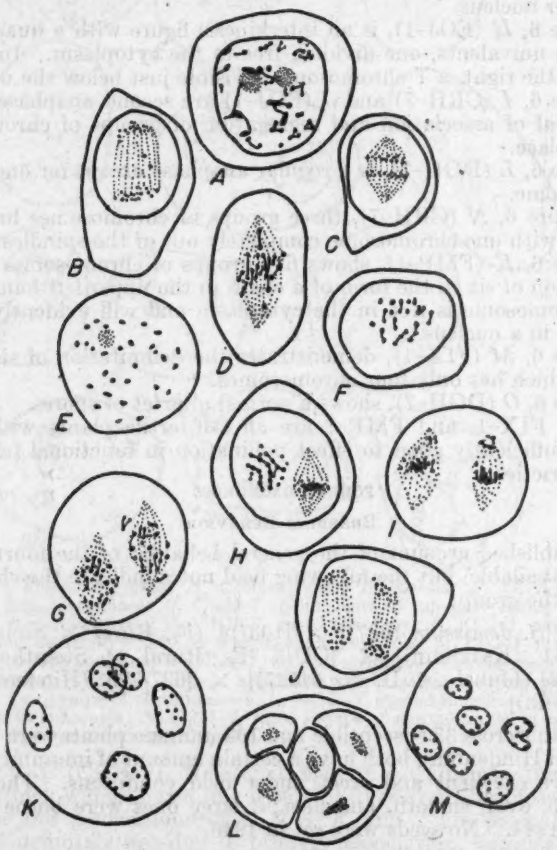

FIGURE 7.—Fourth backcross: *A*, FQE–1, early diakinesis with two nucleoli; *B*, FAU–1, late first anaphase; *C*, FAU–1, regular first anaphase; *D*, FAU–1, first anaphase with associations; *E*, FQE–1, polar view of metaphase; *F*, FNH–4, polar view of first metaphase; *G*, FQE–1, irregular second anaphase; *H*, FAU–1, second metaphase; *I*, FNH–4, irregular second metaphase; *J*, FAU–1, regular second anaphase; *K*, FQE–1, sporocyte with five nuclei; *L*, FNH–4, five spores formed; *M*, FNH–4, six nuclei in sporocyte.

CYTOLOGY

Figure 7, *A*, is an early diakinesis of FQE–1. Two nucleoli are present, with most of the chromosomes appearing as uni-, bi-, and trivalents.

Figures 7, *B* (FAU–1) and *C* (FAU–1) show regular first anaphases, although two chromosomes are lagging in *B*. The absence of associations and the small size of the chromosomes are strikingly evident.

Figure 7, *D* (FAU–1), shows the irregular character of some of the anaphase figures in this same plant. Here are all of the characteristics of the earlier backcrosses.

Figure 7, *E* (FQE–1), is a polar view of a first metaphase in which 30 chromosomes can be counted. No associations are present, and the number is that expected in an F_1 individual.

Figure 7, *F* (FNH–4), is a polar view in which 26 chromosomes, many of them associated, are seen.

Figure 7, G (FQE–1), shows the customary irregular behavior, with associations retained but not in such high numbers as were found in the earlier backcrosses. A trivalent and a bivalent are excluded from the spindle at the right.

Figure 7, *H* (FAU–1), and figure *I* (FNH–4) are of the second division. The polar view at the left in *H* has a high number of associated chromosomes. The chromosomes involved appear to be larger than the unassociated ones. The metaphase figure on the right seems to be proceeding regularly with no associations present. Both spindles in *I* have chromosomes out of line and associations present.

Figure 7, *J* (FAU–1), is a late second anaphase which is proceeding regularly.

Figures 7, *K* (FQE–1), *L* (FNH–4), and *M* (FNH–4) show supernumerary nuclei and pollen grains which result. Normal quartets, however, are quite prevalent in this group of plants.

DISCUSSION

The phenomenon which is most notable in this study of the backcross progeny of a cross between a hexaploid and a tetraploid plant is the continued association of chromosomes found throughout the various stages of meiosis, particularly in the anaphase spindles. In the wild species of *Solanum* with fertile pollen this association is not found, but in the varieties of *S. tuberosum* examined, and in all the hybrids studied, retained anaphase associations and sterility of pollen seem to be directly proportional. If, indeed, in these plants, groups of chromosomes segregate intact, as would appear from the foregoing illustrations, then the number of chromosomes present would give no indication of the ability or inability of the pollen grains to function; it would rather be a matter of which chromosomes are present that would determine functional capacity. Lamm (*8, p. 57*) states: "A comparison between the percentage of balanced II–M plates and the percentage of good pollen shows that some of the apparently good pollen must have an unbalanced chromosome number."

Becker (*1, p. 28*) investigated the chromosome counts of 15 F_2

plants of a *S. demissum* x *S. tuberosum* cross and made the following statement concerning them:

> There was no apparent relationship between chromosome number and either tuber formation or seed production. The approximate chromosome counts of those that set seed as well as tubers were 49, 50, 51, and 56; of those forming only tubers but no seed 50, 53, 54, 56, and 58; and of those forming neither tubers nor seed 48, 52, 53, 54, 56, and 58.

This factor would further explain the seemingly inconsistent behavior of plants from year to year where fertility is concerned. In a pollen-sterile plant some functional grains could be produced, although the odds in its favor would be negligible. Longley and Clark (*11, p. 882*), in their study of chromosome behavior and pollen production in the potato, made the following statement:

> It seems apparent that in different varieties of potatoes the chromosome complement does not show the harmony observed in wild species . . . So pronounced is it [this lack of harmony] in a large percentage of the varieties that there is little or no normal pollen mother-cell development . . . In a very few varieties the chromosome complement seems to be in harmony and the meiotic behavior is almost as regular as that found in material from wild species.

They further state (*p. 883*):

> Although environment may modify somewhat the chromosome behavior during the reduction divisions of the developing pollen mother cells, the writers agree with Fukuda (*5*) [7] that the lack of harmony of the chromosome complement of tetraploid varieties of potatoes is the major cause for the failure to produce, in potatoes, normal 4-cell polen tetrads.

Carrying the situation a bit further, the present writer is of the opinion that the lack of harmony between the chromosome complement and the cytoplasm results in the formation of new connections or in the retention of synaptic associations, which remain intact throughout the divisions, this in turn causing a final unbalance within the chromosome complements in many if not all of the pollen grains. Regardless of regular chromosome numbers and quartet formation, such pollen is functionless. It is, of course, recognized that under these circumstances regular chromosome numbers and quartet formation do not preclude functionless pollen. Becker (*1, p. 27*) found that, although the percentage of stainable pollen for *S. demissum* was 61.3 percent and the mean for the F_1 hybrids was 59.3 percent, the germinated grains in *S. demissum* constituted 26.8 percent, whereas in the hybrids only 2.3 percent germinated. What percentage of these would finally have achieved fertilization in actual tests is not known, but in all probability the percentage would have been lower, judging by the experience of the author and of Reddick,[8] who found that an entire summer spent in hand-pollination of F_1 hybrids of *S. demissum* and *S. tuberosum* resulted in the production of only 98 seeds.

Considering the heterozygous nature of the *S. tuberosum* varieties, a wide range of variation would be expected in the progeny, but such was not the case; most of the plants resembled the *S. demissum* parent, when *S. demissum* was used as the female, and continued to do so in following generations. Obviously the *S. demissum* complement was in rare cases being recovered in both pollen grains and megaspores. Considering the pairing behavior in the F_1 plants investi-

[7] See Literature Cited, reference (*4*), of the present paper.
[8] Oral communication.

gated, wherein synaptic associations of the kinds common to both *S. demissum* and *S. tuberosum* are formed, with no consistent formation of 24 bivalents and 12 univalents observed, a possible explanation may be that pairing inter se of chromosomes derived from each of the parental individuals takes place; that is, that autosyndesis of the *demissum* chromosomes and of the *tuberosum* chromosomes occurs. If such were the case, the *S. demissum* chromosomes functioning in the *S. demissum* cytoplasm would infrequently give rise to a functional grain when by chance they were included in a balanced complement.

This is contradictory to the findings of Salaman (*17, pp. 1238–1239*), who states:

> At the reduction division of the F_1 24 domestic chromosomes units with 24 *S. utile* [*demissum*] chromosomes giving 24 bivalents, the other 12 *S. utile* chromosomes remain unpaired and segregate at random in the heterotype and homotype divisions so that the composition of the pollen-grains varies from 26 to 32. On the assumption that the same type of segregation occurs in the megaspore, mating of gametes would give a variety of forms possessing 24 bivalents + a varying number of univalents. Chance mating of gametes will give two types of chromosome complex in the F_2:
>
> 1. Those containing 60 to 72, i. e., plants derived from gametes containing 6 or more than 6 univalents. . . .
> 2. Those containing from 48 to 60, i. e., derived from gametes containing less than 6 univalents. . . . .
>
> The extra 12 univalents in the F_1 are *S. utile* chromosomes; when 6 or more of them enter the F_2 zygote the individual is *S. utile*-like, in fact it may be suggested that certain of the *S. utile* chromosomes are very stable and that there may be a tendency for gametes possessing many of these extra *S. utile* chromosomes to unite. A pure *S. utile* type of plant is soon extracted from the cross (e. g. in F_2), and the F_4 gives a majority of gametes containing 36 chromosomes (i. e. containing the extra set of *S. utile* chromosomes). The fact that these apparently pure looking *S. utile* plants contain some domestic blood is revealed by the fact that when back-crossed to the parent domestic forms predominantly domestic plants . . . reappear. There is also evidence from the Mendelising of parental characters that the 24 *S. utile* chromosomes and the 24 domestic chromosomes which form bivalents segregate normally, and must not be thought of as segregating in sets of domestic or sets of *S. utile*.

Unfortunately, no illustrations of the cytological features upon which Salaman based his conclusions have been published, and in the material which is here presented, no such regularity as that described by Salaman has been found. There is one initial difference in the material investigated: Salaman used as the pollen parent a "highly inbred domestic seedling," which he says (*17, p. 1236*)—

> has the haploid number 24. . . . The chromosomes are similar in size and shape to those of *S. utile*. The cytology is slightly aberrant, but, compared with the behaviour in some of the domestic varieties examined, it might be characterized as practically normal. Tetrad formation proceeds normally but there is a certain amount of sterile pollen.

There is evidence that in *Solanum* hybrids pairing of chromosomes within a parental complement takes place. Ellison (*3*) reports that in the cross *S. nigrum* ($n\times36$) = *S. nitidibaccatum* ($n\times12$), although bivalents were formed and the divisions were very regular, there was complete sterility. The majority of the pollen grains appeared to be quite normal, but he states (*3, p. 477*): "While normal pollen grains were formed containing 24 chromosomes and the anthers dehisced, the plant failed to set any viable seed under self-pollinating conditions."

In the opinion of the writer there is no way to resolve the dif-

ference of opinion regarding the pairing procedure in *S. demissum* × *S. tuberosum* hybrids except by means of visible markers on the chromosomes that can be followed through synapsis, as has been done in maize.

Secondary associations in the material presented have not been stressed, since there seems to be a difference of opinion concerning the phenomenon (*8, 9, 5*). The associations mentioned in this study refer to actual physical connections and not proximity in position of chromosomes. There is some question as to whether these physical extensions are chromatids that remain unbroken throughout the divisions, or whether, because of "stickiness" of the chromosomes, they reunite at a previous breakage point. In part, the behavior of the hybrids' chromosomes described in this paper are reminiscent of the behavior of the asynaptic diploid and tetraploid plants derived from a colchicine-treated *S. rybinii* chimera described by Lamm (*8*), particularly in the bridge formation and lagging chromosomes. There is some indication that nucleic acid starvation causes stickiness in chromosomes. Lamm (*8, p. 33*) makes the following statement:

> . . . in asynaptic *S. Rybinii* a gene mutation may be responsible for nucleic-acid starvation. At I—M the bridges without fragments could then be of two categories. One of these has already been described, namely, bridge formation due to arrested terminalization in gemini. The other category is thought to be due to sticking univalents. At II—A sister chromatid reunion might be responsible for most of the bridges, this reunion in turn being probably caused by disturbed nucleic-acid metabolism.

This might also be the explanation of the extensions between the chromosomes in some cases where it is quite certain that they were not retained during the divisions.

That the connections are artifacts is in the writer's opinion untenable, since they have been found by cytological investigators in other genera of solanaceous plants (*20*). The regularity with which they are found in spindles of pollen-sterile plants with a corresponding lack in pollen-fertile plants, all of which were given the same treatment, would indicate that they have some significance and are not to be attributed to improper fixation.

In the hybrids discussed in this paper, the stickiness would in all probability not be due to a gene but to the malfunctioning of the chromosomes in the cytoplasm, which might very well result in a disturbance of the nucleic acid metabolism. Reciprocal crosses behave differently in these hybrids, the only unfortunate circumstance being that sterility factors are so prevalent in the Rural varieties that no adequate comparison can be made. Lamm (*7, p. 207*) makes this statement concerning the behavior of reciprocal crosses: "This dissimilar chromosome mechanism in the P. M. C.'s of these *Solanum* hybrids after reciprocal crossings may be due either to cytoplasmic inheritance or to maternal effects."

The T chromosome phenomenon mentioned in the cytological account has been found in *Solanum* species by Lamm (*8*). Prakken and Müntzing (*13, p. 480*) have reported this meiotic peculiarity in rye, which they attribute to "an interaction between the genotypical constitution of the various lines and a structural difference separating the lines with and without a T chromosome pair." The behavior of the T chromosomes simulates the presence of a terminal centromere

in their estimation, since in rye the satellitelike ends of the chromosomes are oriented toward the poles. In potatoes, however, this is not the case, as is pointed out by Lamm (*8, p. 31*):

> As a rule the terminal satellite-like bodies were orientated towards the equator during the first division. In the diploid, out of 64 counted univalents with such bodies, 61 were orientated towards the equator.

The T arm of the chromosome in figure 4, *L*, of this paper is likewise oriented toward the equator.

Taking into consideration all of the abnormalities hitherto outlined, it would seem that the breeding of blight-proof potato varieties by using *S. demissum*×*S. tuberosum* hybrids must remain dependent upon the discretion of the breeder in effecting crosses and then selecting for the characters desired, with little hope of establishing a breeding program wherein expectations, genetically speaking, may be realized. It is very fortunate that any plant having the desired characters can be reproduced asexually in the potato, since in view of the faulty sexual mechanism in the hybrids, it would be impossible to duplicate the specific set of characters from seed.

SUMMARY AND CONCLUSION

The meiotic divisions in the microsporocytes of *Solanum demissum*, *S. tuberosum*, and their hybrid progeny through four backcrosses to *S. tuberosum* have been studied.

Irregularities of meiotic behavior in the hybrids include lagging chromosomes, spindle abnormalities, high multivalent formation, delimitation of fewer or more than four spores, and pollen sterility in most cases. In the divisions resulting in pollen sterility, groups of chromosomes retaining their primary synaptic associations are visible on both anaphase spindles, while in normal polyploid plants segregation of chromosomes in multivalents is at random. Nucleic acid starvation due to the malfunctioning of the chromosomes in the cytoplasm is a possible reason for the retention of associations and bridge formation. These irregularities are found throughout the first-generation hybrids and subsequent backcrosses, but a tendency toward more regularity and greater fertility is expressed in the third and fourth backcrosses. However, the divisions of the third and fourth backcrosses are still highly irregular. and the plants retain many of the undesirable characters of *S. demissum* as well as chromosome numbers which approximate those of the F_1 hybrids. It is suggested that autosyndesis of *S. demissum* and of *S. tuberosum* chromosomes occurs.

The irregularities observed are shown not to be the result of environmental conditions, but to have their origin in constitutional factors involving both the chromosome complement and the cytoplasm.

It is concluded that breeding for blight resistance in commercial varieties of *S. tuberosum* by using *S. demissum*×*S. tuberosum* crosses must remain primarily a trial and error method.

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COMPOSITION OF SHARK MEAL[1]

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INTRODUCTION

Shark meal, a livestock feedstuff high in crude protein content, has been produced in Florida and along the Pacific seaboard in increasing quantities during the past 7 years. This product has proved to be an excellent protein supplement in rations for growing dairy calves (*12*),[3] chicks (*13*), and growing-fattening swine (*14*). Analyses previously reported for shark meal (table 1) show it to be high in crude protein, calcium, and phosphorus. Assays for all of the constituents commonly determined in routine feedstuff analyses have not been reported for any single sample of shark meal.

French[4] stated that shark meal contains some urea nitrogen, which, when calculated as crude protein ($N \times 6.25$), gives a value for the nitrogenous constituents that is excessive. Rhian, Carver, Harrison, and Hamm (*16*) found from 0.09 to 4.37 percent of urea in eight samples of meal made from dogfish sharks. Analyses of these samples by a procedure for determing urea and ammoniacal nitrogen (*3, p. 79*), but omitting the urease, yielded what Rhian et al. termed "nitrogen fraction B" in amounts ranging from 0.35 to 3.55 percent.

Shipments of shark meal received in paraffined containers and paper parcels at the Florida Agricultural Experiment Station had a strong fishy odor in which it was thought that ammonia could be detected. Any loss of nitrogen as ammonia would indicate the presence of it or a precursor in the meal. To acquire more comprehensive information on the composition of shark meal, routine feedstuff analyses were made on 19 samples and spectrographic analyses for the presence of 33 elements were made on the ash of 12. A study was also made of the nature and amounts of nonprotein nitrogen present, and samples were assayed for urease activity.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 218.

[4] French, R. M. Private communication to R. B. Becker, 1942.

TABLE 1.—*Composition of shark-meal samples as reported by different investigators*

Investigator	Processing method	Kind of shark	Analyses							
			Water	Crude protein	Ether extract	Crude fiber	Ash	Ca	P	Chlorine
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Rhian, Carver, Harrison, and Hamm (*16*).	Wet	Dogfish	5.0	67.3	20.8	.....	.....	3.14	2.01	.....
	do	do	4.9	67.2	21.8	.....	10.0	2.29	1.77	.....
	Dry	do	5.0	77.3	13.3	.....	13.3	2.33	1.87	.....
	do	do	11.0	78.5	8.4	.....	11.1	1.76	1.52	.....
	do	do	10.8	77.2	7.1	.....	11.4	1.94	1.78	.....
Evans, Carver, and Hamm (*6*).	Wet	do	2.7	70.8	17.9	.....	9.1	2.30	1.62	.....
	do	do	4.5	73.2	17.4	.....	9.9	2.52	1.73	.....
	do	do	3.4	70.2	20.8	.....	9.7	2.38	1.69	.....
	Dry	do	7.5	82.1	10.5	.....	11.0	2.15	1.74	.....
French [1]	Wet	Unknown	10.6	82.4	6.3	1.2	10.1	.....	1.50	0.46
	do	do	4.8	85.7	.9	.9	14.2	.....	.....	.....
	do	do	8.4	85.6	1.0	1.0	13.3	.....	.....	.03
	do	do	5.1	87.0	1.2	1.8	10.1	.....	.....	.....
	do	do	7.6	78.9	1.4	.9	11.0	.....	.....	.....
	do	do	8.3	80.6	.....	.....	.....	.....	.....	.....
Almquist (*1*)	Unknown	do	.....	74.0	.....	.....	.....	.....	.....	.....
	do	do	.....	81.1	.....	.....	.....	.....	.....	.....
Grau and Almquist (*7*)	do	do	.....	64.3	.....	.....	.....	.....	.....	.....

[1] French, R. M. Private communication to R. B. Becker, 1944.

MATERIAL AND METHODS

Nineteen samples of commercial shark meal manufactured by a wet-processing method (*14*) from sharks caught off the Florida coast during January, February, and March were available for analyses. Moisture, crude protein (N X 6.25), ether extract, crude fiber, ash, and calcium were determined according to the A. O. A. C. (*2*) methods for grain and stock-feed analyses. The phosphorus was determined by the method of Fiske and Subbarow (*6*). Magnesium was determined essentially by the method of Handy (*10*) except that HCl was used and bromcresol green replaced methyl red as the indicator. Nitrogen-free extract was calculated according to the method described by Morrison (*15*). Seventeen of these samples were analyzed for urea by Griem's modification (*8*) of the Griem and Walker method (*9*). Check determinations omitting the urease also were made simultaneously on these samples and the nitrogen values obtained are designated herein as nitrogen fraction A.

Twelve samples of shark meal were analyzed for the presence of 33 elements by the rough estimate method (*17*), using a Littrow type spectrograph. These samples were prepared for analysis by weighing 6 gm. of shark meal into a glazed silica evaporating dish and ashing at temperatures not exceeding 450° C. in a muffle furnace with concealed heating elements. The silica dishes had been leached previously for 24 hours in a mixture of 1 part of HCl, 1 part of HNO_3, and 4 parts of water which had been redistilled in a pyrex glass apparatus. The ash of each sample was placed in a glass bottle, which was sealed with a paper-lined screw top, and transferred to the spectrographic laboratory for analysis.

Tests were made for urease activity on five samples of shark meal. The apparatus used was essentially as described by Hawk and Bergeim (*11*).

Two grams of shark meal, 0.1 gm. of urea, 10 ml. of Clark's (*4*) phosphate buffer solution (pH 6.8), and 150 ml. of water that had been redistilled in a pyrex glass apparatus were placed in a large cylinder which then was stoppered tightly. These materials had been stored previously at a temperature of 40°±1° C. and, after mixing, were incubated at this temperature for 1 hour. Air then was aspirated through this material into 0.1 N HCl for 15 minutes and the excess acid titrated with 0.1 N NaOH. A blank determination with urea omitted was run simultaneously with the assay of each sample.

To study certain changes which might be associated with a loss of nitrogen by volatilization, nitrogen fraction A, urea, pH (using a glass electrode), and moisture were determined on five samples within a few days after processing and again after exposure to the atmosphere for 70 days of storage. The samples were prepared for storage by placing approximately 150 gm. of shark meal in a 400-ml. pyrex glass beaker which then was covered with eight thicknesses of cheesecloth, and set in a room where contamination would be avoided.

EXPERIMENTAL RESULTS

The averages of the analyses for 19 samples of shark meal were: Moisture 9.18 percent, crude protein 78.07, ether extract 2.80, crude fiber 0.32, ash 13.97, calcium 3.48, phosphorus 1.92, and magnesium 0.17 percent. Since the sum of the ingredients for which analyses were made exceeded 100 percent in most cases, calculation of the nitrogen-free extract by difference gave values for only 3 of the samples. Seventeen of these samples contained an average of 1.75 percent of urea and 0.49 percent of nitrogen fraction A. Analyses of each sample together with average and standard error (*18*) are presented in table 2.

TABLE 2.—*Composition of shark-meal samples with average and standard error*

Sample No.	Water	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Ca	P	Mg	Urea	Nitrogen fraction A
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	5.80	83.06	0.12	0.28	1.34	9.40	2.42	1.69	0.16	------	------
2	10.94	82.56	1.58	.37	0	11.66	2.90	1.52	.27	------	------
3	14.95	70.85	4.21	.32	0	13.24	3.19	1.76	.22	1.72	0.490
4	15.34	63.25	3.96	.20	0	19.03	5.24	2.92	.33	1.05	.765
5	14.53	66.04	4.02	.33	0	17.93	4.97	2.60	.25	1.32	.625
6	14.55	68.35	4.09	.19	0	16.46	4.39	2.48	.28	1.48	.680
7	13.83	80.90	4.70	.26	0	16.03	4.33	2.40	.28	1.44	.670
8	7.23	82.12	2.19	.43	0	15.75	3.19	1.72	.13	3.35	.325
9	9.91	87.48	2.78	.17	0	11.28	2.52	1.50	.15	1.25	.465
10	10.04	70.35	4.19	.22	3.49	11.71	2.84	1.60	.20	1.85	.405
11	3.14	79.94	6.44	.20	0	13.76	3.10	1.72	.19	2.51	.365
12	7.58	82.02	2.22	.30	0	12.80	2.87	1.52	.19	.94	.410
13	11.23	77.44	.98	.50	0	14.69	3.89	1.80	.07	2.06	.460
14	4.77	85.50	1.64	.26	0	13.86	3.09	1.76	.06	2.49	.445
15	7.66	82.50	1.39	.57	0	11.97	2.79	1.52	.04	3.16	.270
16	6.36	78.69	.94	.32	0	15.08	3.53	2.04	.06	1.27	.430
17	3.99	88.19	2.48	.45	0	10.85	3.06	1.76	.09	2.00	.495
18	6.49	73.13	2.42	.33	.79	16.84	4.14	2.36	.18	.40	.560
19	6.13	80.94	2.81	.33	0	13.06	3.95	1.76	.06	1.49	.415
Average	9.18	78.07	2.80	.32	------	13.97	3.48	1.92	.17	1.75	.487
Standard error	±.91	±1.67	±.36	±.025	------	±.59	±.19	±.10	±.02	±.19	±.102

The results of the spectrographic analyses on 12 samples of shark meal are presented in table 3. Calcium, phosphorus, sodium, magnesium, silicon, iron, strontium, manganese, zinc, lead, copper, boron, and barium were found in all of the samples. Tin was found in 8 samples, nickel in 3, and chromium in 2. Seventeen of the elements either were absent or present in quantities below the sensitivity range of the method.

TABLE 3.—*Elements detected in shark-meal samples by spectrographic analyses, using the rough estimate method* [1]

(Air-dry basis)

Element [2]	Sample No.												Average
	3	4	8	9	10	11	12	13	14	20	21	22	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Ca	3.4000	4.9350	3.0240	3.0250	1.2830	1.3870	3.1520	3.7320	2.3040	5.3140	4.3850	2.4020	3.1970
P	1.7000	1.9800	3.0240	1.2100	1.2830	1.3870	1.5760	1.8660	1.1520	2.6570	1.7540	1.2010	1.7330
Na	.3400	.5946	.1814	.2420	.2566	.2774	.4728	.3732	.3456	.1594	.3508	.3603	.3303
Mg	.3400	.3964	.6048	.2420	.2566	.4161	.3152	.5598	.1152	.5314	.3508	.2402	.3640
Si	.1700	.1982	.0605	.1210	.0385	.0832	.1576	.1120	.1152	.0797	.1052	.0721	.1094
Fe	.0170	.0198	.0302	.0121	.0128	.0139	.0158	.0187	.0115	.0266	.0175	.0120	.0173
Sr	.0170	.0198	.0302	.0121	.0128	.0139	.0158	.0187	.0115	.0053	.0175	.0120	.0156
Mn	.0005	.0012	.0302	.0363	.0008	.0077	.0009	.0037	.0069	.0008	.0011	.0036	.0078
Zn	.0017	.0059	.0302	.0121	.0128	.0028	.0016	.0112	.0115	.0027	.0018	.0072	.0085
Pb	.0010	.0198	.0030	.0007	.0008	.0008	.0009	.0011	.0007	.0016	.0011	.0007	.0027
Cu	.0010	.0012	.0091	.0007	.0004	.0008	.0009	.0011	.0007	.0016	.0011	.0007	.0016
B	.0005	.0006	.0009	.0004	.0004	.0004	.0005	.0006	.0003	.0008	.0005	.0004	.0005
Ba	.0002	.0002	.0006	.0002	.0003	.0003	.0002	.0002	.0001	.0003	.0002	.0001	.0002
Sn	0	0	.0003	.0001	.0001	.0001	.0002	.0002	.0001	0	0	.0001	------
Ni	0	0	.0005	0	0	0	0	0	.0001	0	0	.0001	------
Cr	0	0	0	0	0	0	0	0	.0001	0	0	.0001	------

[1] The values given indicate that the quantity of a given element is estimated to lie within a range of from one-half to twice the figure given.

[2] Other elements not found in any sample by this method and their lower limits of detectability expressed as percent of the ash are: As, 0.1; Li, 0.03; W and Y, 0.02; Hg, 0.01; Cd, 0.003; and V, La, Co, Zr, Ti, Mo, Be, Bi, Sb, Ge, and Au, each 0.001.

No urease activity was observed in any of the five samples of shark meal tested for the presence of this enzyme.

Some of the nitrogen fraction A was lost during storage from each of the five samples tested. The average value for this fraction on freshly processed samples was 0.394 percent; after 70 days storage it was 0.309 percent when calculated on the basis of the original moisture content. There was no loss of urea during this study. The average

TABLE 4.—*Effect of exposing shark meal to the atmosphere during 70 days of storage on nitrogen fraction A, urea, and hydrogen-ion concentration*

Sample No.	Nitrogen fraction A			Urea			pH		
	Freshly processed	After exposure	Effect of exposure	Freshly processed	After exposure	Effect of exposure	Freshly processed	After exposure	Effect of exposure
	Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*	
8	0.325	0.251	−0.074	3.35	3.36	+0.01	6.05	5.95	−0.10
9	.465	.398	−.067	1.25	1.27	+.02	5.86	5.75	−.11
10	.405	.243	−.162	1.85	1.86	+.01	5.80	5.70	−.10
11	.365	.309	−.056	2.51	2.50	−.01	5.94	5.82	−.12
12	.410	.343	−.067	.94	.95	+.01	5.92	5.80	−.12
Average	.394	.309	−.085	1.98	1.99	+.01	5.91	5.80	−.11

pH of 5.91 for the freshly processed samples decreased to 5.80 during the exposure period. The effect of exposure of shark meal to the atmosphere for 70 days on nitrogen fraction A, urea, and pH is shown in table 4.

DISCUSSION

The total for the percentages of water, crude protein, crude fiber, ether extract, and ash exceeded 100 in many instances, probably because of the inclusion of nitrogen from nonprotein sources in the calculation of crude protein.

The proportion of skeletal material to flesh in the samples was responsible largely for the variation in mineral content and influenced inversely the percentage of crude protein. In the processing of shark meal (*14*) there was little mixing of the material to produce a uniform product. Shark meal is hygroscopic and its moisture content is increased by exposure to a humid atmosphere. Samples 3 through 7, inclusive, were received during very humid weather. The moistureproof bags in which they were shipped were damaged badly during transit and the material had absorbed considerable moisture, as indicated by the analyses (table 2).

The elements determined by the spectrographic method represent those present in the shark-meal samples after they were prepared for analysis. Contamination with some elements possibly could have resulted from contact with the equipment used in the commercial processing of shark meal and in preparation of the samples. The rough-estimate method of determination indicated a range within which the quantity of an element was estimated to lie. The range was from one-half to twice the figure given. This accounted for much of the variation reported in the composition of the samples.

Urease activity of shark-meal origin was not observed, nor was it expected since the product had been subjected to high temperatures during processing (*14*). However, if a large bacterial population were present and conditions were favorable for their growth, urease activity might occur since it has been found (*19*) that many species of bacteria produce this enzyme. Waksman and Davison (*20*) reported that some fungi also possess urease activity.

SUMMARY

The averages of the analyses of 19 samples of shark meal were: Water 9.18, crude protein 78.07, ether extract 2.80, crude fiber 0.32, ash 13.97, calcium 3.48, phosphorus 1.92, and magnesium 0.17 percent. Seventeen of the shark-meal samples averaged 1.75 percent urea and 0.49 percent nitrogen fraction A.

Spectrographic analyses for 33 elements on 12 shark-meal samples showed calcium, phosphorus, sodium, magnesium, silicon, iron, strontium, manganese, zinc, lead, copper, boron, and barium to be present in all samples. Tin was detected in 8 of the samples, nickel in 3, and chromium in 2.

In the 5 samples of shark meal tested for urease activity, none was observed.

The exposure of 5 samples of shark meal to the atmosphere for 70

days did not effect the urea content but resulted in a small loss of nitrogen fraction A and a slight increase in the hydrogen-ion concentration.

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TRANSFER OF THE MOSAIC-RESISTANCE FACTOR BETWEEN H CHROMOSOMES OF NICOTIANA GLUTINOSA AND N. TABACUM[1]

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INTRODUCTION

Mosaic-resistant Holmes Samsoun tobacco was established by substitution of a *Nicotiana glutinosa* chromosome carrying resistance for the H chromosome of Samsoun tobacco. The advantages of this accomplishment from the point of view of the tobacco breeder were, however, marred by inclusion of *glutinosa* factors detrimental to yield linked with resistance in the "alien substitution race." According to Valleau (*8*),[3] several years of backcrossing have failed to separate the resistance factor or factors from those that reduce yield. Evidently failure of pairing of the *N. tabacum* H_t chromosome with its *glutinosa* H_g equivalent in the hybrid prevents exchange of segments and the consequent recombination upon which hopes of improvement rest (for details and references see (*3*)). However, recent evidence, as set forth in the present paper, indicates that an occasional exchange of segments may occur between these two chromosomes.

EXPERIMENTAL DATA

In 1944 seeds of mosaic-resistant burley tobacco which had been derived from Holmes Samsoun by backcrossing to burley and selfing were received from the Plant Industry Station, Beltsville, Md. Three crosses between homozygous resistant plants and either nonresistant burley or the nonresistant variety Purpurea used as a standard at Berkeley were grown from 1944 to 1946. The following cytological observations were made in pollen mother cells of the progeny.

Meiotic behavior was determined in 280 metaphase plates of 3 different crosses, nonresistant×homozygous resistant (table 1). Of these 212 exhibited 24 pairs, while the remainder had an unassociated pair of univalents (or 2 in 2 cases). While it is not possible to identify with certainty a univalent H chromosome because of its similarity

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[3] Italic numbers in parentheses refer to Literature Cited, p. 223.

to other chromosomes of the complement (*2*), the univalents in the $23_{II}+2_{I}$ column of table 1 did not differ perceptibly from the medium large type represented by H. Presumably, then, H is involved in the nonconjunction in all or most cases.

Plant No. 45264p21, which had exhibited no nonconjunction among the small number of PMC's studied, was chosen as the male parent for a cross with a haplo-H plant of the Purpurea variety (table 2). The progeny (46458) segregated independently for haplo and diplo types (37:4 is in fair agreement with the female transmission rate of haplo-H as given by Clausen and Cameron (*2*)) and for mosaic resistance (14 resistant : 25 susceptible fits only poorly the expected 1:1 ratio, $P<.20$). Metaphase plates of selected resistant plants gave the following results (table 2) : All 19 PMC's from 4 different haplo-H plants showed $23_{II}+1_{I}$. Here no nonconjunction was observed among the 23 pairs. But among 18 metaphase figures from 1 resistant diplo-H plant, 46458p28, there were 4 with nonconjunction of H-type chromosomes. Thus the low degree of nonconjunction between the resistance-bearing chromosome and its partner, which was demonstrated in plant 45264p21 and in its siblings, was carried over into the next generation.

TABLE 1.—*Cytology of F_1 nonresistant varieties × resistant burley*

Cross	Population and plant No.	24_{II}	$23_{II}+2_{I}$	$22_{II}+4_{I}$
Burley BR 645 A × resistant burley No. 1.	44180p1	8	1	0
	44180p5	6	1	0
	44180p7	9	0	0
	44180p8	9	11	0
	44180p10	9	0	0
	45264p3	8	2	0
	45264p4	7	0	0
	45264p6	7	0	0
	45264p21	10	0	0
	46454p16	6	2	0
Burley BR 645 A × resistant burley No. 2.	44181p7	4	8	0
	45265p3	0	5	1
	45265p3a	11	0	0
	45265p4	10	0	0
	45265p7	9	1	0
Purpurea × resistant burley No. 1	45266p2	8	2	0
	45266p15	6	4	0
	46455p2	14	4	0
	46455p5	29	1	0
	46455p7	9	6	0
	46455p13	9	3	0
	46455p26	5	4	1
	46455p28	19	11	0
Total		212	66	2

TABLE 2.—*Cytology of Purpurea haplo-H × 45246p21*

Population and plant No.	Number of chromosomes	$23_{II}+1_{I}$	$23_{II}+2_{I}$	$22_{II}+4_{I}$	24_{II}
46458p2a	47	5			
46458p20	47	6			
46458p29	47	2			
46458p36	47	6			
46458p28	48		3	1	[1] 14

[1] Including 1 PMC with 1 very large unconjugated pair resembling the F chromosomes, which occasionally fail to pair in normal material.

Since the H_s chromosome was originally unable to pair with H_t the newly acquired pairing ability must rest upon some modification, presumably a translocation [4] between the H_s and H_t chromosomes, as indicated by the following evidence: (1) The nonconjoined chromosomes in tables 1 and 2 (with the exceptions noted) are of the general type resembling H; this, of course, is not very weighty evidence. (2) The disomic plant No. 46458p28 (table 2), which contained an unmodified H_t chromosome from the Purpurea parent and a modified chromosome from its male parent, showed some nonconjunction. On the other hand, its monosomic siblings with no H_t chromosome derived from the male parent exhibited no nonconjunction of pairs. Thus the H pair was responsible for the nonconjunction observed in the disomic plants.

To prove conclusively that resistance is still carried in an H chromosome and has not been translocated to some other chromosome, a population was grown from a pollination of normal Purpurea with plant 46458p36, one of the resistant haplo-H plants in table 2. If this monosomic plant carried its resistance in some other chromosome, rather than in the H chromosome, its expected progeny should conform to a 1 : 1 ratio. If, however, resistance was located in the H chromosome, then the great majority of the pollen offspring would be resistant. In all the monosomics of *tabacum* tested (*6*) pollen grains with 23 chromosomes showed little ability to compete with those having a full complement. The exact value for the transmission of the monosomic condition has not been determined as yet for the haplo-H type, and upon it depends the frequency of nonresistant plants in the progeny if resistance should be located in H. Also the resistance factor may occasionally become deleted by fragmentation from the H monosome, and again susceptible plants would result. The following values were actually obtained in the experiment: Out of a total of 93 plants (obtained from 130 seeds) 84 proved resistant after inoculation and only 9 were nonresistant. This does not fit a 1 : 1 hypothesis ($P<.01$), even if due allowance be made for seeds which failed to germinate, and the assumption is justified that a modified H chromosome is the carrier of resistance.

DISCUSSION

Modification of chromosomes has been observed frequently before in plants with univalents. Clausen (*1*), Stino (*7*), and Tobgy [5] have described fragmentation and translocation in the progeny of monosomic tobacco.

Several mechanisms have been held in the past to account for translocation. The present data have little bearing on the question whether translocation is a process consisting of two separate steps, namely, breakage followed by fusion of exchanged ends (*5*), or whether it is a result of crossing over between nonhomologous associates (*4*).

[4] "Translocation" is used here to designate an exchange between the two nonpairing chromosomes; the term is usually defined more specifically as an attachment of a sector from one chromosome to a nonhomologue.

[5] TOBGY, A. H. A CYTOGENETIC STUDY OF SOME CHROMOSOMAL ALTERATIONS IN A MONOSOMIC TYPE OF NICOTIANA TABACUM, 96 pp. 1946. [Unpublished doctoral thesis. Copy on file in library, University of California, Berkeley.]

The fact that no metaphase conjugation has been observed between H_g and H_t (*3*) lends more support to the breakage-fusion theory for this specific case, but it is inconclusive. If, on the other hand, conjugation occurs at all, it should bring into contact not only nonhomologous sectors, but also homologous regions of the H_g and H_t chromosomes.

It has been argued (*3*) that the H_g and H_t chromosomes are of similar genetic composition. H_g can replace H_t in miscrospores as well as in somatic tissues, although these cannot function without H. Plants containing the alternates cannot be distinguished, except by quantitative measurements. The absence of pairing was assumed, therefore, to be caused mainly by the differences in the linear sequence of the loci. A large number of inversions was believed to have caused the differentiation between H_g and H_t during the phylogeny of the parental species. Thus any pairing between the chromosomes might bring into contact short homologous sectors in which chiasmata could be formed.

Though it is desirable for the breeder to break up the H_g linkage group and to extract the resistance factor (or factors) alone, the processes just mentioned involve a difficulty which should not be overlooked. Crossing over between fully homologous chromosomes is an orderly process, causing no loss or addition of loci, while these types of exchange happen in a hit-or-miss fashion and the resulting chromosomes may well be deficient for some loci necessary to a "normal" tobacco plant. Deficiencies may result either from translocation in two separate steps or from crossing over between two structurally differentiated chromosomes. This difficulty cannot be avoided; after the exchange has taken place the translocated strain must be tested. Deficiencies for essential genes should produce some of the morphological features of the haplo-H type when heterozygous, and weakness or even lethality when homozygous. Such a test has not yet been undertaken in the present material.

This study cannot be regarded as terminated before a number of questions are resolved. The data obtained do not make possible a decision as to the relative length of the *tabacum* and *glutinosa* segments in the translocated chromosome. Presumably the *glutinosa* segment includes at least an entire arm, as otherwise pairing should be even more complete than the data in table 1 indicate. No data on chiasma frequency in Purpurea are available, but nonconjunction is a rare event in normal plants. If, therefore, the two chromosomes in question had more than a complete *tabacum* arm in common in the heterozygote, they would almost always pair in at least one arm. Some further information as to the relative lengths could be gained from a study of the degree of conjugation in a hybrid between Holmes Samsoun (with an entire H_g chromosome) and a plant containing the translocation. If the above estimate is correct, H_g should pair with the translocated chromosome in virtually every cell.

Finally it remains to be determined whether the translocation has eliminated the *glutinosa* factors that reduce yield and whether it is possible to narrow down further the *glutinosa* sector till only the resistance factor (or factors) is retained. This latter goal could presumably be attained by further translocation or crossing over

between the *tabacum* and the *glutinosa* sectors. Some indication that this occurs lies in the variation in the degree of nonconjunction between the plants listed in table 1. This variation may be due to differences in the composition of the modified chromosomes carried by these plants (see especially Nos. 46455p5 and 46455p28). If such differences have already become established further reduction of the length of the *glutinosa* segment may be possible.[6]

SUMMARY

The "alien substitution race" Holmes Samsoun and its derivatives which carried mosaic resistance in a pair of *Nicotiana glutinosa* chromosomes substituted for the H pair of *N. tabacum* have proved somewhat unsatisfactory to breeders because *glutinosa* factors which reduce yield were linked with resistance. Recombination did not occur because of the failure of the *glutinosa* chromosome to pair with its *tabacum* partner in heterozygotes. This paper discusses the transfer of a segment carrying resistance from the *glutinosa* chromosome to the H chromosome of *tabacum*, which is the initial step towards separation of the desirable *glutinosa* factors from the undesirable ones.

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between the tobacco and the glutinous sectors. Some indication that this occurs lies in the variation in the degree of nonsegmentation between the plants listed in table 1. This variation may be due to differences in the composition of the modified chromosomes carried by these plants (see especially Nos. 4114-5295 and 4114-5295). If such differences have already become established further reduction of the length of the glutinous segment may be possible.

SUMMARY

The Italian substitution race, Holmes Samsoun, and its derivatives, which carried mosaic resistance in a pair of N. tabacum plus chromosomes substituted for the H pair of N. tabacum have proved somewhat unsatisfactory to breeders because glutinous factors which reduce yield were linked with resistance. Recombination did not occur because of the failure of the glutinous chromosome to pair with its tabacum partner in heterozygotes. This paper discusses the transfer of a segment carrying resistance from the glutinous chromosome to the H chromosome of tabacum, which is the initial step towards separation of the desirable glutinous factors from the undesirable ones.

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